CANADIAN JOURNAL OF RESEARCH

VOLUME 17 SEPTEMBER, 1939 NUMBER 9

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NATIONAL RESEARCH COUNCIL
OTTAWA, CANADA

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A and B	$ 2.50	$ 0.50
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Four sections, complete	4.00	—

The Canadian Journal of Research is published by the National Research Council of Canada under authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. All correspondence should be addressed:

National Research Council, Ottawa, Canada.

Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 17, SEC. C. SEPTEMBER, 1939 NUMBER 9

A MUTATION FOR PATHOGENICITY IN *PUCCINIA GRAMINIS TRITICI*[1]

BY MARGARET NEWTON[2] AND THORVALDUR JOHNSON[3]

Abstract

A pathogenic change, explainable only on the assumption of mutation, has occurred in a uredial culture of race 52 of *Puccinia graminis Tritici*, which had previously remained constant in pathogenicity for nearly two years. The mutation appears to have taken place during a six-month period of storage of the urediospores in a refrigerator maintained at a temperature of about 8° C. When cultured in the greenhouse, at the end of this period, the rust appeared to be a mixture of race 52 and a hitherto undescribed physiologic race, with the latter predominating. The original culture was left in storage for a further period of four months, after which it gave rise to a pure culture of the new race without any indication of the presence of race 52. The new race has been assigned the number 178.

Although physiologic races of the various cereal rusts appear for the most part to remain constant in their pathogenic properties year after year, there have been reports, nevertheless, of sudden changes (mutations) in spore colour or pathogenicity. In *Puccinia graminis Tritici* Erikss. & Henn., mutation for uredial colour has been reported by Waterhouse (7) and by Newton and Johnson (3), while mutation for pathogenicity has been recorded by Stakman, Levine, and Cotter (6). In other cereal rusts, evidence for mutation has been presented by Johnston (2) and by Roberts (5) for *Puccinia triticina* Erikss.; by Gassner and Straib (1) for *Puccinia glumarum* (Schmidt) Erikss. & Henn.; and by d'Oliveira (4) for *Puccinia anomala* Rost.

In the greenhouse studies, the present writers have occasionally noticed apparently spontaneous pathogenic changes involving partial or entire displacement of one physiologic race by another. Owing to the fact that such changes have usually given rise to races already known and studied in the greenhouse at some previous time, it has never appeared safe to attribute them to mutation. Recently, however, a physiologic race has undergone a pathogenic change in which the original race was displaced by one hitherto undescribed. In this instance it does not appear possible to explain the change except by assuming that mutation occurred.

[1] *Manuscript received May 30, 1939.*

Contribution No. 589, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada. (Continuing the series of the former Division of Botany.)

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The pathogenic change occurred in a culture of race 52 derived from a selfing* of race 56 originally collected at Arnes, Manitoba, in 1934. In the selfing study, which was carried out in the fall of 1935, 21 uredial cultures, each arising from a single aecium, were identified. Culture No. 8 was identified as race 52 on January 8, 1936, and was placed in storage in a refrigerator kept at a low temperature (about 8° C.) on March 3. It was removed from the refrigerator in October of the same year and was cultured in the greenhouse until December 28 when it was again stored in the refrigerator after its purity (as race 52) had been checked. It was again brought into the greenhouse in the spring of 1937, checked for purity and placed in storage on May 17. Part of the stored rust material was brought into the greenhouse once more in November, 1937. The infection types then produced on the differential wheat varieties indicated that race 52 had been largely replaced by another race, although the presence, on infected leaves of the variety Vernal, of a few large pustules characteristic of race 52 among the smaller pustules of some other race showed that the replacement was not complete. Attempts to recover race 52 by inoculating seedling wheat leaves with spores of these large pustules were unsuccessful owing to failure of infection. It is therefore not absolutely certain that the large pustules just referred to were actually those of race 52, although the likelihood is that they were. The remainder of the culture, on further study, proved to be a hitherto undescribed physiologic race with infection types differing widely from those characteristic of race 52, as is shown by the following comparison.

	LC*	Ma.	Krd.	Ko.	Arn.	Mnd.	Spm.	Kub.	Ac.	Enk.	Ver.	Kpl.
New race	4**	1	0;	0;	4	x−	x	x+	4	3+	0;	1
Race 52	4	4	4−	4	1=	1=	1=	x±	4	4−	4+	1−

* *Explanation of abbreviations:* LC = *Little Club*, Ma. = *Marquis*, Krd. = *Kanred*, Ko. = *Kota*, Arn. = *Arnautka*, Mnd. = *Mindum*, Spm. = *Spelmar*, Kub. = *Kubanka*, Ac. = *Acme*, Enk. = *Einkorn*, Ver. = *Vernal*, Kpl. = *Khapli*.

** *Explanation of symbols representing infection types:* 0; = *hypersensitive flecks*, 1 = *minute pustules surrounded by necrotic areas*, 3 = *pustules of moderate size*, 4 = *large pustules*, x = *pustules of various sizes on the same leaf.* (+) (±) (−) (=) *indicate variations in pustule size.*

In an attempt to recover race 52, inoculations were made four months later from the remaining spore material in storage in the refrigerator. The culture thus established proved to be a pure culture of the new race without any indication whatever of the presence of race 52. After some further study of the new race, its mean infection types were submitted to Dr. E. C. Stakman and Dr. M. N. Levine, who kindly numbered it physiologic race 178.

* *A selfing involves (i) inoculating plants of Berberis vulgaris with sporidia of a pure culture of a physiologic race, (ii) intermixing the nectar of the pycnial pustules, (iii) inoculating wheat plants with the aeciospores, and (iv) identifying the physiologic races in the uredial cultures thus established.*

The evidence for mutation in this instance rests on the facts (i) that the culture was placed in storage immediately after its purity as race 52 had been established, (ii) that while in storage there was no opportunity for contamination by another physiologic race, and (iii) that race 52 was replaced by a race not hitherto known. The fact that the original race was replaced by a race never before encountered appears to eliminate the possibility of contamination, which is the only alternative to mutation.

This instance of the replacement of one physiologic race by another suggests a series of recurrent, identical mutations similar to those described by Gassner and Straib (1) in *Puccinia glumarum*. It is clear, at any rate, that more than one urediospore was involved in mutation as the new race was obtained from the stored spore material on two separate occasions. The fact that race 52 was not recovered the last time the culture was re-established in the greenhouse does not, however, necessarily imply that all the stored spore material had become converted into the new race. The infection results obtained at that time suggested that only a small fraction of the stored spore material was viable. It is quite possible, therefore, that all the spores of the original race had been rendered non-viable by the long period of storage and that only a few spores of the new race remained germinable and capable of causing infection.

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ESTIMATION OF LEAF AREA IN WHEAT FROM LINEAR DIMENSIONS[1]

By J. W. Hopkins[2]

Abstract

Measurements of 80 to 90 leaves of each of four varieties of spring wheat at various stages of development indicate a fairly close statistical relation between area, and length and width, of the leaf blade. This relation was found to be essentially the same for all four varieties, and from a knowledge of length (L) and median width ($W_{\frac{1}{2}}$), the area of an individual leaf was given by the Least Squares relation log. $A = 0.0094 + 0.934$ log. $L + 1.071$ log. $W_{\frac{1}{2}}$, with a standard error of 4.2% of the antilog. Inclusion of a third measurement, width at three-quarters of the distance from base to tip ($W_{\frac{3}{4}}$), led to the relation log. $A = -0.0438 + 0.970$ log. $L + 0.880$ log. $W_{\frac{1}{2}} + 0.189$ log. $W_{\frac{3}{4}}$, giving estimated values having a standard error of 3.7% of the actual area per leaf.

This method of estimating leaf areas (i) is rapid in execution, and (ii) does not necessitate removing the leaves from experimental plants, which may accordingly be maintained intact for a series of physiological observations.

Introduction

In the study of certain types of plant physiological data, such as transpiration and growth rates, it is necessary to determine, or at least to estimate with reasonable accuracy, the leaf area of the experimental plants. It may be noted that it is customary to make comparisons relative to the area of the leaf laminae only, although in plants of the Grass family the leaf sheaths, which may attain considerable dimensions, are also functional in both transpiration and metabolism.

Four categories of methods for estimating the area of leaf laminae may be recognized. (i) Reproduction of the outline of each leaf by tracing, blueprinting, sensitized paper or similar means, and determination of the area thus delineated either directly or by weighing. This was the procedure employed by Brown and Escombe (1) in their classical studies of foliar metabolism, and is still used extensively. (ii) Use of an apparatus based on the photoelectric principle, the direct readings of which may be calibrated in terms of area. Problems and recent progress in this connection have been described by Kramer (3). (iii) By computation from the correlation between leaf area and leaf weight. This correlation is the basis of an indirect method of estimating the leaf area of field crops due to Watson (4). His procedure is to determine the mean leaf weight from a large random sample of plants, then to determine the leaf area : leaf weight ratio and its regression on leaf weight, from a relatively small subsample of single leaves. (iv) By measurement of linear dimensions, from which the area may be approximately computed. This was the method employed by Clements and Goldsmith (2) in their quantitative studies of ecological factors, using plants as measuring instruments. These authors conclude that the average area of both sides of a grain

[1] *Manuscript received June 9, 1939.*
Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Published as N.R.C. No. 836.
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leaf is 1.5 times the product of the extreme length and width, but do not indicate the order of accuracy to be expected from this approximation.

It will be appreciated that methods (ii), (iii), and (iv), are all more rapid than (i), but (ii) and (iii) both still retain the disadvantage that the leaves must be removed from the plant, which is consequently destroyed for further experiments. On the other hand, measurements of length and breadth, which may be made *in situ*, are not only equally rapid, but also leave the plant intact. As, however, the leaves of Grasses do not present regular geometrical figures, the relation between area and any specified linear dimensions must be a statistical one. The observations reported below were accordingly made in order to provide some information respecting the degree of precision to be expected in practice from the estimation of areas in this way.

Data and Results

Plants of four varieties of spring wheat, namely, Marquis, Reward × Caesium, Caesium × Marquis, and Lutescens, were grown in a greenhouse during the winter months from seed kindly supplied by Professor K. W. Neatby, of the University of Alberta. Specimens of each variety were collected at regular intervals for the measurement of length, width, and area of leaves at successive stages of growth. Length of leaf blade from base to tip was measured to the nearest $\frac{1}{2}$ mm., using a good quality steel ruler. Width at the mid-point, and also at a point three-quarters of the distance from base to tip, was measured to the nearest $\frac{1}{10}$ mm. by indicating calipers. Areas were determined by a Rotometer. In all, between 80 and 90 leaves of each variety were examined.

In general outline the wheat leaf is long and narrow, tapering to a point at the tip. As a rule, however, the width of the blade at first increases somewhat as one proceeds upwards from the base until a maximum is attained which, in the case of full-grown specimens, is generally in the lower half of the leaf. The shape of the lower part of the blade is therefore approximately rectangular, or more correctly, trapezoidal, whilst the tapering upper part (neglecting the peculiar but characteristic constriction occurring at a point usually about $1\frac{1}{2}$–2 in. from the tip) presents approximately the aspect of an isosceles triangle, of which the base is small in relation to the perpendicular height. From geometrical considerations, therefore, a linear relation between area and the product of length and median width might be anticipated, which, however, would be expected to be only imperfectly realized in practice, owing to individual variations in the relative proportions of the trapezoidal and triangular portions of the leaf, to a certain degree of curvature in outline, and to occasional outright irregularities in shape.

Fig. 1, in which the measured area of 89 Lutescens leaves is shown plotted in relation to the product of length and median width, indicates that this is the situation actually prevailing.

Fig. 1 also illustrates the fact that irregular deviations are greater in the case of large than of small leaves. A logarithmic transformation tends to

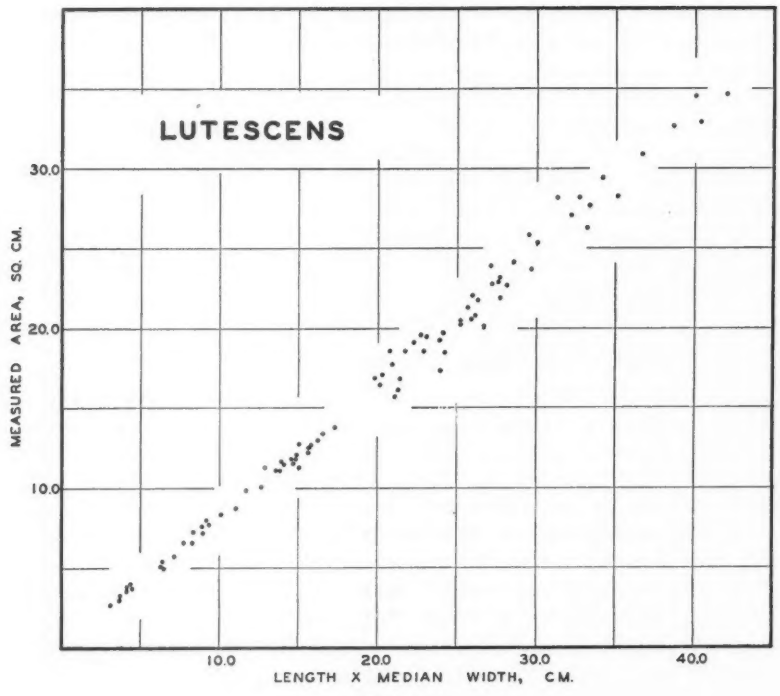

FIG. 1.

stabilize the variance in these circumstances, and also facilitates further analysis. The linear relation between area (A) and the product of length and median width ($L \times W_{\frac{1}{4}}$) was accordingly determined in logarithmic units for each of the four varieties, and was found to be:

Marquis:	$\log. A = -0.0533 + 0.990 \log. (L \times W_{\frac{1}{4}})$
Reward × Caesium:	$\log. A = -0.0560 + 0.986 \log. (L \times W_{\frac{1}{4}})$
Caesium × Marquis:	$\log. A = -0.0652 + 0.995 \log. (L \times W_{\frac{1}{4}})$
Lutescens:	$\log. A = -0.0290 + 0.984 \log. (L \times W_{\frac{1}{4}})$

The standard errors of estimation of log. A by these formulae are 0.0167, 0.0224, 0.0199, and 0.0197, respectively, or 3.9, 5.3, 4.7, and 4.7% of the antilog.

During the course of the measurements, it was observed that when the median width was above average, the point of maximum width tended to occur further up the leaf, resulting in an increase of the approximately rectangular relative to the triangular portion, and hence in a proportionately greater area.

This impression was confirmed by the computation of the regression of log. A on log. L and log. $W_{1/2}$ separately, which gave:

Marquis	log. A =	$0.0106 + 0.932 \log. L + 1.074 \log. W_{1/2}$
Reward × Caesium:	log. A =	$0.0105 + 0.936 \log. L + 1.060 \log. W_{1/2}$
Caesium × Marquis:	log. A =	$-0.0199 + 0.927 \log. L + 1.118 \log. W_{1/2}$
Lutescens:	log. A =	$0.0699 + 0.909 \log. L + 1.073 \log. W_{1/2}$

The difference between the regression coefficients for log. L and log. $W_{1/2}$ is statistically significant, and as a result of taking this circumstance into account, the residual standard error of log. A is reduced to 0.0140, 0.0215, 0.0176, and 0.0179, or 3.3, 5.1, 4.1, and 4.2% of the antilog. for the four varieties respectively.

A still further increase in precision was obtained by utilizing the additional information provided by the width at three-quarters of the distance from the base to the tip of the leaf ($W_{3/4}$). When this was done, the Least Squares solutions for the regression equations became:

Marquis:

$$\log. A = -0.0354 + 0.962 \log. L + 0.921 \log. W_{1/2} + 0.153 \log. W_{3/4}$$

Reward × Caesium:

$$\log. A = 0.3580 + 0.794 \log. L + 0.826 \log. W_{1/2} + 0.245 \log. W_{3/4}$$

Caesium × Marquis:

$$\log. A = -0.0479 + 0.965 \log. L + 0.909 \log. W_{1/2} + 0.182 \log. W_{3/4}$$

Lutescens:

$$\log. A = -0.0030 + 0.954 \log. L + 0.874 \log. W_{1/2} + 0.196 \log. W_{3/4}$$

These give standard errors of estimation of 0.0120, 0.0187, 0.0157, and 0.0145 logarithmic units, or 2.8, 4.3, 3.7, and 3.4% of the actual area of an individual leaf.

In order to determine whether the inter-varietal differences in the regression coefficients of area on length and width were statistically significant, the standard errors of the individual coefficients were calculated. In the case of the first approximation, namely, the regression of area on the product of length and median width, the regression coefficients and their respective standard errors in logarithmic units, are:

Marquis:	0.990 ± 0.0045
Reward × Caesium:	0.986 ± 0.0069
Caesium × Marquis:	0.995 ± 0.0073
Lutescens:	0.984 ± 0.0074

The differences between the varietal coefficients are not significant in relation to their standard errors.

The coefficients and their standard errors (again in logarithmic units) in the case of the regression of area on length (L), median width ($W_{1/2}$) and width three-quarters of the distance from base to leaf-tip ($W_{3/4}$) are:

	L	$W_{1/2}$	$W_{3/4}$
Marquis:	0.962 ± 0.010	0.921 ± 0.030	0.153 ± 0.027
Reward × Caesium:	0.974 ± 0.017	0.826 ± 0.051	0.245 ± 0.039
Caesium × Marquis:	0.965 ± 0.016	0.909 ± 0.050	0.182 ± 0.039
Lutescens:	0.954 ± 0.016	0.874 ± 0.034	0.196 ± 0.029

There is no significant difference between the varietal regression coefficients in respect of either L or $W_{1/2}$. In the case of $W_{3/4}$, the coefficient for Reward × Caesium is slightly higher than those for the other three varieties, which do not differ significantly amongst themselves.

In these circumstances, it would seem that little additional error would be introduced by the use of an average regression equation. The data for the four varieties were accordingly pooled and used to determine such equations for two of the three approximations considered above, it having been found already that better results were obtained by the inclusion of L and $W_{1/2}$ separately than by the use of the product $L \times W_{1/2}$.

In this way, the average regression of A on L and $W_{1/2}$ was found to be:

$$\log. A = 0.0094 + 0.934 \log. L + 1.071 \log. W_{1/2}$$

with a residual standard error of estimation of A of 0.0182 logarithmic units, or 4.2% of the antilog.

Including $W_{3/4}$, the solution for the average regression equation was:

$$\log. A = -0.0438 + 0.970 \log. L + 0.880 \log. W_{1/2} + 0.189 \log. W_{3/4}.$$

This was found to have a residual standard error of estimation of A of 0.0158 in the logarithm, or 3.7% of the antilog., which may be regarded as satisfactory for the purpose in view.

The foregoing results cannot be compared directly with those of Clements and Goldsmith (2), but it may be noted that whereas they found 0.75 for the mean ratio of area to length × extreme width, the mean measured area of the 89 Lutescens leaves in Fig. 1 is 1619 sq. mm., and the mean product of length × median width 1960, giving a ratio of 0.86. It is evident, therefore, that the agreement in this respect must be fairly close.

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EFFECTS OF PLANT AND ANIMAL HORMONES ON THE ROOTING OF DUST- AND SOLUTION-TREATED DORMANT STEM CUTTINGS[1]

By N. H. Grace[2]

Abstract

Indolylbutyric acid and oestrone were applied in dusts to dormant stem cuttings of *Lonicera tartarica*, *Spiraea Vanhouttei*, and *Cornus alba*, and in both dusts and solutions to cuttings of *Ribes odoratum*. Indolylbutyric acid had significant effects on the number of cuttings rooted and the number and length of roots per rooted cutting of three species. Further observations indicated that it also affected the fresh root weight of cuttings of *Spiraea Vanhouttei* and the green leaf weight of *Ribes odoratum*. Oestrone had no significant effect on rooting, but in solution treatment showed significant effects on the green leaf weight of *Ribes odoratum*, both alone and in interaction with indolylbutyric acid. Cuttings of *Cornus alba* failed to show any significant treatment effects.

Dust and solution methods of treating cuttings were compared through the responses of *Ribes odoratum*. Dust treatment effected 62% rooting, solution 42%; there also was markedly greater leaf development following the use of dusts.

Oestrogenic substances have been shown to occur in plant materials, and their effects on plant growth have been reported by several investigators (1, 2, 4–10, 13). It has been concluded that some of these substances may have to be considered as plant hormones (11). While the influence of these chemicals on the rooting of cuttings has not been reported in detail, it is suggested that the number of roots produced by auxin treatment is increased by oestrogenic materials (12). The present communication describes the results of experiments in which dormant cuttings were treated by talc dusts and solutions of indolylbutyric acid and oestrone.

Experimental

Dust and solution preparations were compared, the indolylbutyric acid and oestrone being used at the same concentration; the interaction between chemicals was investigated by one series of solution treatments. Dusts containing 2000 p.p.m. of chemical in talc were prepared in a small laboratory ball mill. Subsequently, these were mixed with talc to give 1000 and 500 p.p.m. concentrations. Oestrone* solution was prepared by dissolving 0.0700 gm. in 3 cc. of warm 95% alcohol and making up to a volume of 350 cc., which gave a 200 p.p.m. colloidal solution. This solution was stirred continuously while aliquots were withdrawn. Indolylbutyric acid solution contained the same amount of alcohol.

Dormant cuttings of current year's growth of *Cornus alba* L., *Spiraea Vanhouttei* Zabel, *Lonicera tartarica* L. and *Ribes odoratum* Wendl. were

[1] *Manuscript received July 12, 1939.*

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Issued as N.R.C. No. 837.

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* *Theelin was provided through the kindness of Dr. Oliver Kamm, Parke Davis Co., Detroit.*

collected in mid-December, 1938*. The cuttings of the first three species ranged from 10 to 12 in. in length; those of *Ribes odoratum* approximated 6 in., with two buds. Treated cuttings were planted in brown sand in a propagation frame equipped with electrical bottom heat cables. Sand temperature was maintained around 72° F., while the greenhouse temperature approximated 65° F.

The dust series of experiments contained seven treatments, namely, talc only, and 500, 1000, and 2000 p.p.m. of each chemical separately in talc. There were 10 cuttings to a group and 7 replicates of each treatment; 490 cuttings were required for each experiment.

Lonicera tartarica cuttings were left for 24 days in sand. After determination of the number of cuttings rooted and the number and length of roots, the basal 2 in. of each cutting was removed and the entire experiment replanted in sand in random order, in the hope that residual treatment effects might be demonstrated. The cuttings were examined 31 days later, to determine whether treatment with oestrone or indolylbutyric acid had an effect on the development of a second growth of roots by the shortened cuttings. Cuttings of *Cornus alba* were examined 28 days after planting. *Spiraea Vanhouttei* cuttings were inspected 48 days after treatment.

Cuttings of one plant, *Ribes odoratum*, were treated with both dusts and solutions and were removed from the propagation frame 47 days after planting. The two experiments were placed contiguously in the same frame, thus permitting a rough comparison of the responses following treatment by two different methods of applying the chemicals. The solution experiment was of factorial design, which permitted study of the interaction of indolylbutyric acid and oestrone. There were four concentrations of the two chemicals, namely, 0, 10, 50, and 100 p.p.m. This made a series of 16 treatment combinations, with 10 cuttings to a group and four replicates of each treatment.

The design of the experiments provided for analyses of variance of the observations. Record was made of the number of cuttings rooted and the number and length of the primary roots formed. The mean length of root was calculated. In one experiment, where secondary root formation was pronounced, the roots were carefully washed in running water, placed between dry sheets of blotting paper for five minutes, and fresh root weights recorded. The new green growth produced by these dormant cuttings was also removed and weighed.

Results

Responses after Dust Treatment

In Tables I to III, data are given for responses after dust treatment with indolylbutyric acid and oestrone; residual effects are discussed separately.

Highly significant increase in rooting is shown by all three concentrations of indolylbutyric acid applied to cuttings of *Lonicera tartarica*, whereas the

* *The prepared cuttings were supplied by the Federal District Commission through the kindness of Mr. E. I. Wood.*

corresponding concentrations of oestrone had no demonstrable effect. Only the means over the three concentrations of indolylbutyric acid and oestrone give significant results with *Spiraea Vanhouttei*; the indolylbutyric acid mean is significantly above that for oestrone but is not above the talc-treated control. Treatments failed to affect the number of *Ribes odoratum* cuttings rooted and the data are not given.

Oestrone treatments failed to have any effect on the number of cuttings rooted.

TABLE I

RESPONSES OF DORMANT *Lonicera tartarica* CUTTINGS DUSTED WITH PLANT AND ANIMAL HORMONES, AND RESIDUAL EFFECTS FOLLOWING REMOVAL OF BASAL ENDS

Data are means for seven groups of ten cuttings

—	Talc dusted	Dusted with indolylbutyric acid in talc, p.p.m.			Dusted with oestrone in talc, p.p.m.			Necessary difference, 5% level
		500	1000	2000	500	1000	2000	
Number of rooted cuttings								
Transformed data*	1.92	2.86**	2.84**	3.06**	1.83	1.97	1.75	0.36
Untransformed data	3.4	7.7	7.6	8.9	3.0	3.6	2.7	
Number of roots per rooted cutting								
Transformed data*	1.99	3.08	3.53**	3.88**	2.05	1.98	2.00	0.45
Untransformed data	3.7	9.0	12.1	14.9	3.9	3.6	3.7	
Total root length per rooted cutting, mm.	63	245**	326**	337**	100	81	86	89
Residual effects								
Number of rooted cuttings	6.3	4.3	5.6	5.1	5.9	5.0	3.9	—
Number of roots per rooted cutting	9.3	10.3	6.8	9.3	10.8	8.6	11.3	—
Total root length per rooted cutting, mm.	277	295	206	258	394	255	348	—
Mean length per root, mm.	30	30	27	26	36	31	30	—
Green leaf weight, gm.	6.2	6.0	6.4	6.1	7.7	6.7	6.5	—

* *Data transformed to* $\sqrt{x + \frac{1}{2}}$ *basis (3).*

** *Values significantly different from the talc dusted control.*

Treatment with indolylbutyric acid had significant effects on the number and lengths of root per rooted cutting in all species; oestrone treatment had no significant effect. Data on the mean length of root failed to attain significance for individual treatments. Partition of the treatment sum of squares for the data on *Ribes odoratum* indicated a lengthening effect over the mean of all treatments.

TABLE II

RESPONSES OF DORMANT *Spiraea Vanhouttei* CUTTINGS DUSTED WITH PLANT AND ANIMAL HORMONES

Data are means for seven groups of ten cuttings

—	Talc dusted control	Dusted with indolylbutyric acid in talc, p.p.m.				Dusted with oestrone in talc, p.p.m.				Necessary difference, 5% level
		500	1000	2000	Mean for all indolyl-butyric treatments	500	1000	2000	Mean for all oestrone treatments	
Number of rooted cuttings										
Transformed data*	2.446	2.344	2.653	2.514	2.503	2.270	1.953	2.095	2.107	0.276†
Untransformed data	5.7	5.1	6.6	5.9	5.8	4.9	3.6	4.3	4.2	
Total root length per rooted cutting, mm.	380	508	711**	425	548	400	455	477	434	191 111†
Fresh weight of roots, gm.	1.04	1.74	2.54**	1.24	1.84	1.03	0.66	1.09	0.93	0.73 0.43†

* *Data transformed to* $\sqrt{x + \frac{1}{2}}$ *basis (3).*

** *Values significantly different from the talc dusted control.*

† *This necessary difference holds between means by chemicals; 0.39 is the necessary difference between control and group means.*

TABLE III

RESPONSES OF DORMANT *Ribes odoratum* CUTTINGS TREATED WITH PLANT AND ANIMAL HORMONE DUSTS

Data are means for seven groups of ten cuttings

—	Talc dusted control	Dusted with indolylbutyric acid in talc, p.p.m.			Dusted with oestrone in talc, p.p.m.			Necessary difference, 5% level
		500	1000	2000	500	1000	2000	
Number of roots per rooted cutting	3.61	4.57	5.69**	7.07**	3.54	3.64	2.87	1.12
Total root length per rooted cutting, mm.	88	149	187**	254**	153	136	94	68.6
Mean length per root	25	33	32	35	42	36	33	
Mean for six treatments with two chemicals		35**						7.9*
Green leaf weight, gm.	2.2	2.5	2.1	1.4**	2.3	2.1	2.0	0.60

* *Necessary difference comparing talc dusted control and mean for the six dust treatments which do not vary significantly among themselves.*

** *Values significantly different from the talc dusted control.*

The 1000 p.p.m. treatment with indolylbutyric acid had a significant effect on the fresh root weight of *Spiraea Vanhouttei*; no other root weights were determined. No significant effects could be attributed to oestrone.

One significant treatment effect is shown by the green leaf weight of *Ribes odoratum* cuttings. The 2000 p.p.m. indolylbutyric treatment, which gave more roots per rooted cutting than any other, shows markedly reduced leaf growth. It would seem therefore that marked stimulation of the rooting response may be accompanied by reduced leaf development.

Cuttings of *Cornus alba* failed to show any significant treatment effects.

In the second part of Table I are given data on the residual effects of plant and animal hormone treatments on the various responses of *Lonicera tartarica* cuttings. While differences are suggested by the data, analyses of variance show that treatments are insignificant in each instance. Partition of the treatment sum of squares also failed to indicate any significant effects. It may be concluded therefore that removal of the basal end of the cutting, which takes away all roots on rooted cuttings and the zone which has been dust-treated, leaves a cutting whose further responses are not affected by the initial treatment.

Responses after Solution Treatment

In Table IV are given data for the responses of solution-treated *Ribes odoratum* cuttings. It is apparent that the 50 p.p.m. treatment with indolylbutyric acid gives optimum rooting in the series, the response falling off at the 100 p.p.m. level. Indolylbutyric treatment also effects marked increase in both the number and length of roots per rooted cutting. Oestrone treatment, however, fails to show any significant increase in rooting over the

TABLE IV

RESPONSES OF DORMANT *Ribes odoratum* CUTTINGS TREATED WITH SOLUTIONS OF INDOLYLBUTYRIC ACID AND OESTRONE

Data are means for sixteen groups of ten cuttings

	Indolylbutyric concentrations, p.p.m. (average over all oestrone concentrations)				Oestrone concentrations, p.p.m. p.p.m. (average over all indolylbutyric concentrations)				Necessary difference, 5% level
	0	10	50	100	0	10	50	100	
Number of rooted cuttings									
Transformed data*	1.86	2.08	2.36**	2.13	2.18	2.30	1.95	2.0	0.273
Untransformed data	3.2	4.1	5.2	4.2	4.3	4.9	3.6	3.8	
Number of roots per rooted cutting	4.1	3.9	6.2**	6.6**	4.9	5.6	5.2	5.1	1.32
Total root length per rooted cutting, mm.	113	143	203**	213**	148	177	182	166	61

* *Data transformed to* $\sqrt{x + \frac{1}{2}}$ *basis (3).*

** *Values significantly different from the respective control.*

control, although the 50 p.p.m. treatment is significantly below the 10 p.p.m. level; the interaction between indolylbutyric acid and oestrone is insignificant.

The data in Table V indicate that treatments have a highly significant effect on the weight of leaf produced. Both indolylbutyric acid and oestrone treatment, and the interaction between them, are significant. Leaf weight falls with increasing indolylbutyric concentrations, the values at 50 and 100 p.p.m. both being significantly below the 0 p.p.m. value. The 10 p.p.m. oestrone treatment is significantly above the 0 value, stimulating leaf development. There is significant depression at the 50 p.p.m. level and stimulation again as the 100 p.p.m. concentration is reached. The two stimulating concentrations, 10 and 100 p.p.m., do not differ between themselves, while the depressing 50 p.p.m. level differs significantly from the other three concentrations. The interaction effect would seem to be a consequence of the fact that both the stimulatory and depressive effects of oestrone are more pronounced at the 0 and 10 p.p.m. levels of indolylbutyric acid than when applied in conjunction with 50 or 100 p.p.m. of the latter substance.

TABLE V

GREEN WEIGHT OF LEAVES PRODUCED BY DORMANT CUTTINGS OF *Ribes odoratum* FOLLOWING TREATMENT WITH SOLUTIONS OF INDOLYLBUTYRIC ACID AND OESTRONE, IN GRAMS

Data are means for four groups of ten cuttings

Oestrone concentrations, p.p.m.	Indolylbutyric acid concentrations, p.p.m.				Means for oestrone treatments
	0	10	50	100	
0	1.03	0.73	0.70	0.43	0.72
10	1.50	1.15	0.80	0.58	1.01
50	0.65	0.40	0.58	0.35	0.49
100	0.88	1.18	0.90	0.60	0.89
Means for indolylbutyric treatments	1.01	0.86	0.74	0.49	
Necessary difference, 5% level: treatment means, 0.16; interaction, 0.32					

Comparison of Responses of Ribes odoratum Cuttings Following Dust and Solution Treatment

Incidentally, a comparison has been made of the dust and solution methods of treating cuttings through the responses of *Ribes odoratum*, averages over all treatments being considered. Since these two methods of applying physiologically active chemicals are in general use, comparison of the results is of considerable interest. In Table VI are given data for the responses following these two methods of treatment. Dust treatment effected 62% rooting, solution treatment 42%. This is of interest since the treatment effect failed to attain significance for the number of cuttings rooted after dust treatment. The absence of significant treatment effects after dust treatment may be attributed to the rooting of the talc-treated control. Solution treatment tends to produce somewhat more roots per rooted cutting. A striking difference is noted in the weight of green growth after dust treatment; this is

nearly three times that found on solution-treated cuttings. These differences would suggest somewhat greater physiological shock from solution treatment or a beneficial effect from the talc carrier.

TABLE VI

RESPONSES OF *Ribes odoratum* CUTTINGS FOLLOWING TREATMENT WITH DUSTS AND SOLUTIONS OF INDOLYLBUTYRIC ACID AND OESTRONE

Results for dust treatment are means of 490 cuttings, solution treatment means of 640 cuttings

	Dust method of treatment	Solution method of treatment
Number of rooted cuttings of 10 planted	6.2	4.2
Number of roots per rooted cutting	4.4	5.2
Total root length per rooted cutting, mm.	151.4	167.9
Mean root length, mm.	33.8	32.2
Green leaf weight, gm.	2.09	0.78

Acknowledgment

The author wishes to express his appreciation to Dr. H. A. Senn, Botany Division, Department of Agriculture, for checking the identification of the plants used.

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VEGETATIVE PROPAGATION OF CONIFERS

II. EFFECTS OF NUTRIENT SOLUTION AND PHYTOHORMONE DUSTS ON THE ROOTING OF NORWAY SPRUCE CUTTINGS[1]

By N. H. Grace[2]

Abstract

Norway spruce cuttings were treated with phytohormone dusts, and nutrient solution was added to the sand in which some of the cuttings were planted. The nutrient treatment greatly increased the number of rooted cuttings and the number that developed new growth, and reduced the number that died. Although talc alone increased top growth, indolylacetic acid, present in three concentrations in talc, had no significant effect on the number of cuttings rooted or dead. However, the hormone dust treatment effected a significant reduction in the length of root per rooted cutting and the mean root length. The results indicate that nutrient salts may, under certain conditions, have a marked influence on the rooting and growth of Norway spruce cuttings.

While the importance of phytohormone chemicals in propagation is recognized, it is clear that other factors have a profound influence on the rooting of conifer cuttings. It has been demonstrated that the age of the tree, the position on the tree from which the cutting is taken, and the stage of development, are factors that affect rooting (2, 3, 7). Recent work by Nowasad has shown that nutrient solutions increase the rooting of alfalfa cuttings (5, 6). The present communication describes the results of an experiment at the National Research Laboratories, Ottawa, in which nutrient solution was applied to the sand in which a series of hormone-dust-treated Norway spruce cuttings were planted.

Experimental

The experiment was carried out in a propagation frame, which contained 5 rows of 12 glazed earthenware crocks provided with suitable bottom drainage and filled with washed brown sand over a layer of coarse sand. The crocks were set in sand heated by electrical cables.

There were five treatments:— untreated, talc only, and 5, 100, and 1000 p.p.m. of indolylacetic acid in talc. The experiment was arranged in the form of double blocks of 10 crocks. Each block contained two replicates of the dust treatments, one which received water only, the other nutrient solution weekly. The random arrangement gave the greatest precision to the comparison between nutrient and no nutrient treatments.

The nutrient solution used was Hoagland's; to it was added approximately 120 p.p.m. sodium chloride, 1 p.p.m. boron, 0.44 p.p.m. manganese and 0.16 p.p.m. zinc (4). Each of the 30 crocks receiving nutrient was given 200 cc. of this solution weekly for seven weeks. The solution was washed down

[1] *Manuscript received July 13, 1939.*

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry, N.R.C. No. 839.

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immediately with about 200 cc. of tap water. The rest of the crocks were given an equal amount of water at the same time. All the crocks were kept suitably moist by application of tap water as required. The pH of the nutrient solution was about 5.1, that of the sand immediately after treatment and washing down with tap water, 7.4. At the end of the experiment, four months after planting the cuttings, there was no significant difference in pH of the sand from crocks that had, and those that had not, received nutrient, the pH ranging around 8.1. The alkaline condition must be attributed to the Ottawa tap water, the pH of which ranges between 8 and 9.

Branches from the upper region of the tree were collected in January from a plantation of Norway spruce approximately 18 years of age and situated at the Dominion Forest Station, Chalk River, Ontario. The bases of the branches were covered with moist peat and held about two weeks in a room at 65° F. Cuttings were torn from the branches and trimmed with a knife, leaving a heel of old wood; they were divided into two classes, first, those from 10 to 20 cm. in length, and second, those from 5 to 10 cm. Each group of 15 contained 10 short and 5 long cuttings. There were approximately four laterals to one branch terminal. Groups of 15 were dust treated and planted immediately. The entire propagation frame was covered with a factory cotton screen. The temperature of the sand was maintained at 72° F. and that of the room between 65 and 75° F. However, after one month under these conditions, outside temperatures rose rapidly. It was impossible to keep down the room temperature which frequently rose to 90° F. or even higher during the latter part of the experiment.

Seven weeks after planting it was observed that most of the crocks were infected with an unidentified fungus. The incidence and extent of the infection did not appear related to nutrient treatment; nevertheless, this was discontinued. Each of the 60 crocks was treated with 100 cc. of a 2 p.p.m. ethyl mercuric bromide solution, and the treatment repeated twice at intervals of three days†. The organic mercurial application completely eliminated the infection and, apparently, had no detrimental effect on the cuttings.

Results

Cuttings were taken up four months after planting and a record was made of the number rooted, showing new growth, and dead, in each replicate of 15. The number of roots and their lengths were determined and the mean root length was calculated for cuttings that received nutrient. As only five cuttings rooted in the 30 crocks that did not receive nutrient, data on number and lengths of roots could not be analyzed for this part of the experiment. The data thus secured were subjected to analyses of variance, with the results indicated in Table I.

† *The ethyl mercuric bromide used in this experiment was prepared by a method developed in the Chemistry Division, National Research Laboratories, Ottawa, by Dr. A. Cambron, in the course of an investigation of the synthesis of alkyl mercury halides. The procedure followed yielded a product consisting of 80% ethyl mercuric bromide and 20% ethyl mercuric chloride.*

TABLE I

ANALYSIS OF VARIANCE OF RESPONSE OF NORWAY SPRUCE CUTTINGS TO PHYTOHORMONE DUSTS AND NUTRIENT SOLUTIONS

Source of variance	Degrees of freedom	Mean square					
		Number of cuttings‡			Mean, per rooted cutting		Mean root length, mm.
		Rooted	Dead	With top growth	Number of roots	Length of roots, mm.	
Replicates	5	76.60	43.52	46.30	0.225	1617.2	638.51*
Treatments	4	143.24	119.64	150.23*	0.280	2601.4*	577.47*
Error *(a)*	20	66.54	54.78	44.98	0.164†	696.1†	163.27†
Nutrient concentration	1	7898.24***	1847.04***	7346.05***			
Interaction of treatments and nutrient	4	29.49	53.03	116.90	§	§	§
Error *(b)*	25	73.99	57.25	62.36			

‡ *Data transformed from fractions to degrees (1).*

† *Eighteen degrees of freedom for error as two missing values have been estimated (8).*

§ *As only five cuttings rooted without nutrient treatment, data on number and length of roots could not be analyzed for that part of the experiment.*

* *Exceeds mean square error, 5% level of significance.*

*** *Exceeds mean square error, 0.1% level of significance.*

The data in Table II compare the effects of nutrient and no nutrient on the cuttings rooted, showing new top growth, and dead. The upper half of the table gives percentages of the number of cuttings alive at the end of the four months in sand. The lower half gives percentages of the total cuttings planted. The data are presented in this manner because virtually 100% of

TABLE II

RESPONSES OF NORWAY SPRUCE CUTTINGS TREATED WITH NUTRIENT SOLUTIONS, FOUR MONTHS AFTER PLANTING

——	Treatment with	
	Nutrient solution	Water only
Living cuttings rooted, %.....	37.5	2.9
Living cuttings with new growth, %	31.8	1.2
Cuttings rooted, %†	20.7	1.1
Cuttings with new growth, %	17.6	0.4
Cuttings dead, %	44.9	61.8

† *Lower half of table gives percentages of all cuttings planted.*

the cuttings from 10 to 20 cm. in length died. It is apparent that weekly application of nutrient solution increased the number of cuttings that rooted and that showed new growth; there is, also, a highly significant reduction in the number of cuttings that died. It is of interest to point out that, of the nutrient-treated cuttings alive at the end of the experiment and without new growth, 38.5% were rooted, whereas 35.4% of the cuttings with new growth were rooted. Consequently, it is suggested that development of top growth by Norway spruce cuttings does not cause any marked reduction in the initiation of roots.

In Table III are given data for the effect of dust treatment on the number of cuttings with top growth, the length of root per rooted cutting, and mean root length. It may be seen from Table I that these are the only significant effects of dust treatment. Talc is significantly more effective than other

TABLE III

EFFECT OF HORMONE DUST TREATMENTS ON THE NEW GROWTH AND ROOT LENGTH OF NORWAY SPRUCE CUTTINGS

——	Untreated	Talc	Indolylacetic in talc, p.p.m.			Necessary difference, 5% level
			5	100	1000	
Number of cuttings having top growth, transformed data†	9.5	17.6	10.3	13.6	9.3	5.71
Per cent of planted cuttings showing new growth	6.7	15.6	5.6	10.6	6.6	
Root length per rooted cutting, mm.*	74.2	95.2	60.2	56.3	39.7	31.99
Mean root length, mm.*	44.0	54.0	41.7	44.7	26.8	15.50

† *Data transformed from fractions to degrees (1).*

* *Root lengths refer to cuttings which received nutrient solution.*

treatments in producing cuttings with top growth; the effects of other treatments differ only slightly among themselves. The greatest length of root per rooted cutting occurs on talc treatment, the results of which are significantly better than those of all three hormone dust treatments but not significantly better than those obtained with the untreated control. The 1000 p.p.m. level of indolylacetic acid reduces the mean root length. While the talc treatment gives the greatest mean length, the results do not differ significantly from those of the untreated control or the 5 and 100 p.p.m. levels. This reduction of mean root length of Norway spruce cuttings with a heel when treated with 1000 p.p.m. indolylacetic acid in talc has been reported previously (3). It is apparent that treatment with indolylacetic acid over the range from 5 to 1000 p.p.m. in talc has failed to stimulate the responses of Norway spruce cuttings; as little as 5 p.p.m. in the dust has reduced significantly the development of top growth.

The results indicate that nutrient treatment has a marked beneficial effect on the rooting of Norway spruce cuttings. In another experiment, the results of which will be published in due course, nutrient solution was used as the carrier for the hormone chemical but beneficial response was not indicated. It is possible that the high temperatures, the incidence of fungal infection, or initial storage of the branches in this experiment, are factors that accentuated damage and emphasized the effect of treating sand with nutrient. In consequence, it cannot be assumed that such marked stimulation of response would follow the use of nutrient treatment of the sand under growth conditions more nearly optimum. However, it is apparent that the judicious use of nutrient salts may present an important aid to the development of well rooted, vigorous conifer cuttings.

The absence of positive effects from indolylacetic acid treatment is noteworthy. The improvement from treatment with talc only is of interest and suggests that dusting of cuttings is advantageous. However, considerable work will be required before any definite mechanism for the effect of talc on cuttings can be established.

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Canadian Journal of Research

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 17, SEC. D. SEPTEMBER, 1939 NUMBER 9

STUDIES ON THE BIONOMICS AND CONTROL OF THE BURSATE NEMATODES OF HORSES AND SHEEP

VII. THE EFFECT OF SOME SUBSTANCES, USED IN THE CONTROL OF FARM AND HOUSEHOLD PESTS, ON THE FREE-LIVING STAGES OF SCLEROSTOMES[1]

BY I. W. PARNELL[2]

Abstract

The effect on the free-living stages of Sclerostomes caused by the addition to fresh horse faeces of some substances used in the control of household and farm pests, is discussed. Under the conditions of these experiments para- and ortho-dichlorbenzene will sterilize about 400 times their weight of faeces. Sodium fluoride will sterilize, on an average, approximately 150 times its weight of faeces, but it is almost twice as effective if applied as a very weak solution. Sodium silicofluoride, which also is most effective as a very weak solution, probably has an approximately equal value. Naphthalene, when mixed in the faeces, will sterilize about 270 times its own weight. Dichloropentanes will sterilize about 185 times their weight of faeces. 40% nicotine sulphate will, on an average, sterilize approximately 14 times its weight of faeces, but as a weak solution may be five or six times as effective. Ethylenedichloride, chloroform and carbon tetrachloride will sterilize about 21, 18 and 12 times their weight of faeces respectively. Trisodium phosphate will sterilize only about eight times its weight of faeces. Tobacco dust will probably sterilize slightly over twice its weight of faeces, but pyrethrum powder, derris powder and white hellebore powder have no lethal value. Ferric oxide and carbon monoxide also are useless.

This paper discusses the lethal effect of: paradichlorbenzene, orthodichlorbenzene, sodium fluoride, sodium silicofluoride, naphthalene, dichloropentanes, 40% nicotine sulphate, ethylenedichloride, chloroform, carbon tetrachloride, trisodium phosphate, tobacco dust, pyrethrum powder, derris powder, white hellebore powder, ferric oxide, and carbon monoxide, against the free-living stages of Sclerostomes in faeces, when the faeces were treated before the eggs could develop, according to the technique described in previous papers (22, 23).

Only under exceptional conditions is it probable that many of these chemicals could be used to control the free-living stages of Sclerostomes or related worms, in practice. However, it is hoped that some of the results may be of value in indicating some of the types and states of chemicals, which are, or are not, lethal to the free-living stages of this group of worms.

Dichloropentanes are practically the only chemicals of the above list that have been recommended, even tentatively, for use in Sclerostome control (16).

[1] *Manuscript received June 28, 1939.*

Contribution from the Institute of Parasitology, McGill University, Macdonald College, Que., with financial assistance from the National Research Council of Canada.

[2] *Lecturer in Parasitology.*

Ethylenedichloride and carbon tetrachloride, and a mixture of the two at the rate of 3 : 1 by volume, have also been tested in horse faeces (16). The addition of from 0.5 to 0.8 cc. of dichloropentanes per 100 gm. of faeces reduced the number of larvae in the cultures to 0.0006% of the number in the controls, or less. When 0.7 to 0.9 cc. of ethylenedichloride and of carbon tetrachloride were added to the same quantity of fresh faeces the numbers of larvae were also very considerably reduced. Alone, carbon tetrachloride at the rate of slightly under 1 cc. per 100 gm. was effective; ethylenedichloride was probably slightly less effective. These three chemicals were apparently more effective against the eggs and pre-infective larvae than against the infective larvae.

Some of the chemicals that are discussed in this paper have been tested against nematodes that parasitize plants; in a few cases, in spite of the difficulty of comparing the results, they are suggestive.

Paradichlorbenzene at the rate of 2½ oz. per sq. yd. has failed against *Heterodera marioni* of tomatoes (24). A similar result was obtained against *H. schachtii* of potatoes in pots (1); however, on a larger scale, at the rate of 616 lb. per acre, an increased yield of potatoes resulted, although the nematodes were little affected (7). It has also been tested against fly larvae in manure at the rate of ½ lb. and 1 lb. per 10 cu. ft. of manure, but was not very effective (4). Against wireworms in soil the results with this chemical are contradictory (9). Its effectiveness against clothes moths, etc., is well known, and it is occasionally used by gardeners in greenhouses against slugs and snails. It acts on the nervous system of insects (5). The nervous system of Sclerostome larvae is very rudimentary, although sufficient to make them react quite quickly to various stimuli, such as light, heat, and touch. Orthodichlorbenzene has been tested less frequently.

The usefulness of sodium fluoride and sodium silicofluoride in farm practice is limited by their poisonousness to mammals. Sodium fluoride in solution of 1 and 2 lb. per gal. has been tested against fly larvae in manure; at the rate of 1 gal. to 1 cu. ft. of manure it was fairly effective (2). However, a 2% solution of sodium fluoride and a saturated solution of sodium silicofluoride had no effect after two hours on *Tylenchus dipsaci* (19).

Naphthalene also has been tested fairly frequently against plant nematodes and again the results are contradictory. Some workers have obtained no effect (1, 6, 13, 24); others have obtained slightly better results (17, 18). Against wireworms the results also are contradictory (9), but it is well known for its action against clothes moths.

Nicotine sulphate has been shown to be effective against fly larvae in manure, even as a 1 : 500 solution (4). As a constituent of sprays for fruit trees, as a fumigant for poultry lice, and as an anthelmintic, it is well known. Against free-living nematodes it has seldom, if ever, been tested.

Ethylenedichloride is more effective than carbon tetrachloride against *Tylenchus dipsaci*. If one part of the latter is mixed with three parts of the former, the fire hazard is reduced. Ethylenedichloride rapidly produces

anaesthesia, but a longer exposure (2 hr.) is necessary to cause the death of the nematodes (20). However, against nematodes in bulbs, ethylenedichloride was not really effective as a controlling agent (21).

Pyrethrum, derris, and hellebore powders are well known for their lethal action on certain insects. Against the leaf eelworm of chrysanthemums, pyrethrum and nicotine emulsions were more or less unsuccessful (15). Against fly larvae, hellebore is more effective powdered than ground (4), and as an infusion than as a powder (3).

The results obtained when ferric oxide has been applied to potatoes and oats have been very variable; under certain conditions the results have appeared to be beneficial (8, 12), and under others it has not been effective or has only temporarily stimulated the plants (7, 10, 11, 14).

Results

Table I shows the values of the "controls" which are identified in Figs. 1 to 11 by Roman numerals.

TABLE I

CONTROLS FOR CULTURES TABULATED IN FIGS. 1 TO 11

Series No.	Date cultures made	Days kept in C.T. room	Average number of larvae isolated	Series No.	Date cultures made	Days kept in C.T. room	Average number of larvae isolated
	1935				*1937—Conc.*		
XIII	7 May	32	11,000	CCLXIII	15 July	71	33,000
XXIX	12 July	20	19,500	CCLXV	19 July	67	18,000
XXXVII	6 August	35	38,000	CCLXVI	19 July	77	41,000
LIV	10 December	17	6,200	CCLXX	22 July	74	34,000
	1936			CCLXXVI	1 October	24	66,000
				CCLXXVII	6 October	29	26,500
LXXIV	24 January	24	11,500	CCLXXXIII	19 October	27	34,000
LXXXIII	3 March	13	25,000	CCLXXXIV	19 October	27	65,000
CXLI	9 July	24	10,000	CCXC	28 October	28	46,000
CLVII	28 October	37	43,000	CCXCI	29 October	38	67,000
CLX	2 November	42	15,500	CCIC	16 November	42	53,000
CLXVI	16 November	45	23,500	CCCII	23 November	35	24,500
CLXXII	24 November	51	35,000	CCCIII	24 November	44	32,000
CLXXIII	26 November	60	37,000	CCCVI	2 December	36	63,000
CLXXXIV	17 December	56	52,000	CCCVII	3 December	35	58,000
CLXXXVI	28 December	49	42,000	CCCVIII	3 December	45	48,000
	1937			CCCIX	8 December	40	39,000
				CCCX	9 December	39	28,500
CCII	29 January	48	19,000	CCCXIII	20 December	38	34,000
CCIII	2 February	52	27,000		1938		
CCX	23 February	44	35,000				
CCXI	24 February	50	32,000	CCCXVIII	3 January	35	30,000
CCXIV	3 March	43	41,000	CCCXXVII	1 February	37	35,000
CCXV	3 March	47	45,000	CCCXXVIII	2 February	36	29,000
CCXVIII	11 March	60	39,000	CCCXXIX	3 February	35	40,000
CCXLI	18 May	27	49,000	CCCXXX	25 February	87	12,500
CCXLII	18 May	27	35,000	CCCXXXI	7 March	77	19,500
CCXLIII	18 May	27	91,000	CCCXXXVII	28 March	70	19,000
CCVLII	1 June	23	66,000	CCCXXXVIII	28 March	84	14,500
CCVLIII	1 June	23	85,000	CCCLVI	6 July	51	38,000
CCLVIII	12 July	64	43,000	CCCLVII	7 July	50	32,000

Paradichlorbenzene

Paradichlorbenzene was tested both in the faeces and suspended in cheese cloth bags above them; Fig. 1 illustrates the results. Quantities of 0.01 to 8.0 gm. were applied. The cultures with the paradichlorbenzene above them showed that only about 1.7 gm. would evaporate in the containers, which have a capacity of nearly 550 cc. The results, like those obtained with some other chemicals that give off gases, were very irregular.

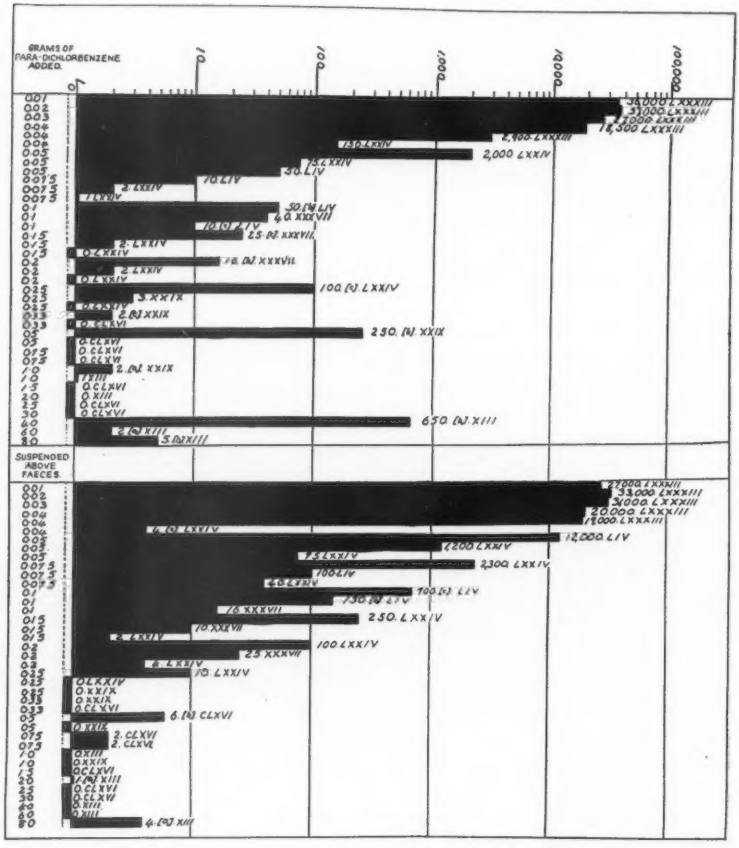

FIG. 1. *Results of tests with paradichlorbenzene, mixed in and suspended above the 40-gm. cultures of fresh horse faeces. Roman numerals refer to the controls shown in Table I. In this, and in the subsequent figures, the letters have the following significance. a, all or practically all these Sclerostome larvae were dead; b, a considerable proportion of larvae were dead; c, a few larvae were dead; d, the culture included some Sclerostome larval sheaths or other debris, which was not counted; e, in the culture there were some live larvae, not counted, some or all of which were probably exsheathed Sclerostomes and which were sufficiently numerous to have put the culture in a significantly more numerous class, if they had been counted.*

The number of larvae in one culture was considerably reduced by 0.04 gm. and in two cultures by 0.05 gm. mixed in the faeces. When 0.075 gm. was used, only 1, 2, and 10 larvae were recovered. With the addition of 0.1 gm. the larvae were slightly more numerous, but some were dead.

When suspended above the faeces, 0.04 and 0.05 gm. considerably reduced the number of larvae in one culture each, and 0.075 gm. reduced them in two cultures. From the cultures treated with 0.1 gm., 700, 150, and 15 larvae were isolated, but some were dead; 0.15 gm. reduced the larvae to 250, 10, and 2, while 0.2 gm. reduced them to 100, 25, and 4.

An average of these results suggests that under these conditions about 0.1 gm. or slightly less will sterilize the 40-gm. cultures of fresh horse faeces against Sclerostomes; this is equivalent to 0.25% of the weight of faeces.

Orthodichlorbenzene

Orthodichlorbenzene, which has a specific gravity of 1.325, was tested in quantities of from 0.01 to 25.0 cc. Fig. 2 shows its sterilizing value. Again the results are very irregular. The number of larvae was considerably reduced by 0.033 cc. and the cultures were almost sterilized by 0.05 and 0.075 cc.; only four, three, and no larvae were recovered from the cultures treated with 0.1 cc. All the cultures treated with 0.15 and 0.2 cc. were free of larvae.

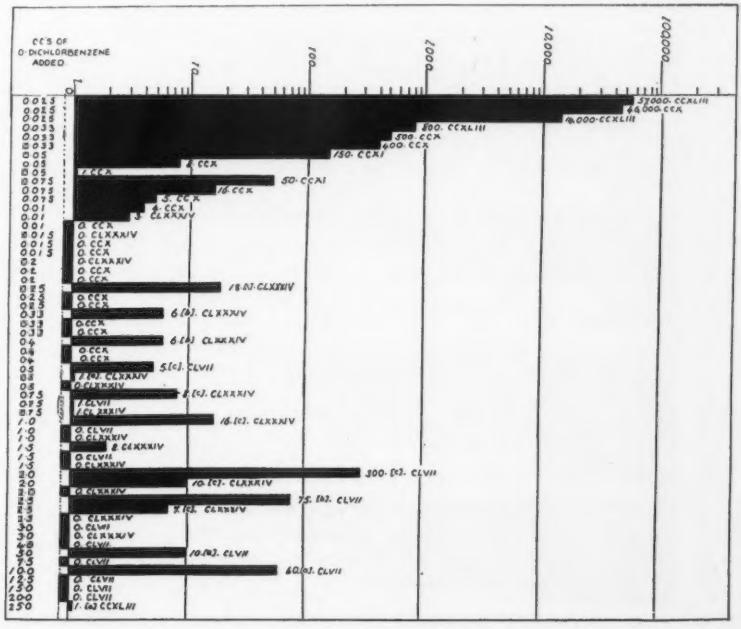

FIG. 2. *Results of tests with orthodichlorbenzene.*

Among the few larvae that were recovered from the cultures treated with larger quantities, the death rate was high. The amount of orthodichlorbenzene equivalent to about 0.25% of the weight of the treated faeces is 0.075 cc.

Sodium Fluoride

Fig. 3 illustrates the results obtained with sodium fluoride when applied dry or in solution.

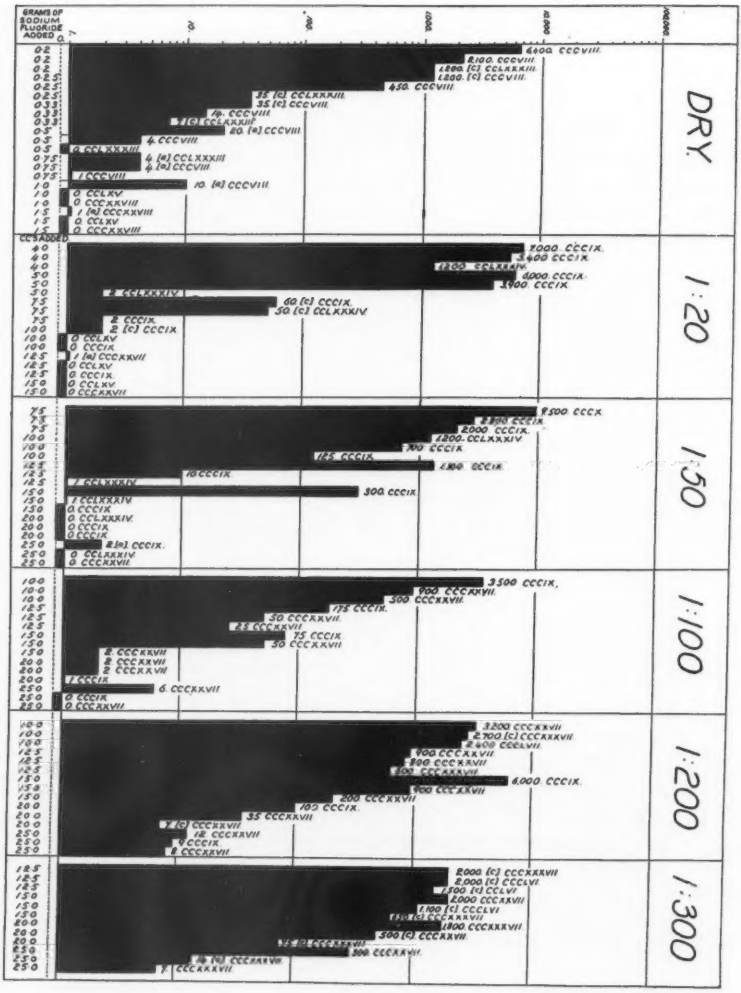

FIG. 3. *Results of tests with sodium fluoride, dry and in solution.*

It was applied dry in quantities of 0.05 to 8.0 gm. When 0.2 gm. or less was applied, thousands of larvae survived. The numbers were considerably reduced by 0.25 gm.; 0.33 gm., or 0.82% of the weight of faeces, was practically effective as a sterilizing agent. No larvae were found in any of the cultures treated with 2.0 gm. or over.

As a 1 : 20 solution, quantities of 2.0 cc. and over were tested. With one exception, thousands of larvae were recovered from the cultures treated with 5.0 cc. or less; 7.5 cc. was practically and 10.0 cc. was completely effective. In 10.0 cc. of a 1 : 20 solution there is almost 0.5 gm. of sodium fluoride, equivalent to 1.25% of the weight of faeces.

As a 1 : 50 solution, which was tested in quantities of 4.0 cc. and over, 10.0 cc. made a very noticeable reduction in the number of larvae, while 12.5 cc. and 15.0 cc. both sterilized two out of three cultures; all the cultures were sterilized by 20.0 and by 25.0 cc. In 15.0 cc. of a 1 : 50 solution there is approximately 0.3 gm. of sodium fluoride, or 0.75% of the weight of faeces.

Applied as a 1 : 100 solution, 10.0 cc. again considerably reduced the number of larvae; 12.5 and 15.0 cc. reduced them further, and the latter quantity sterilized one culture. Sterilization was almost complete when 20.0 and 25.0 cc. were applied; the former quantity contains 0.2 gm., or 0.5% of the weight of faeces.

Sodium fluoride was also tested as a 1 : 200, 1 : 300, 1 : 400, 1 : 500, and 1 : 600 aqueous solution. Fig. 3 shows that the greater quantities of fluid of both the 1 : 200 and 1 : 300 solutions caused a marked reduction in the number of larvae; the other solutions, which are not illustrated, had similar, but less marked, effects on the numbers. The 1 : 300 and 1 : 400, like the 1 : 200, solutions, tended to cause the death of a few of the larvae after they had reached the third stage. In 25.0 cc. of a 1 : 200 solution there is about 0.125 gm. of sodium fluoride and only about 0.083 gm. in the same quantity of fluid of a 1 : 300 solution; these quantities are equivalent to 0.31% and 0.21% of the weight of faeces. If the different quantities of sodium fluoride that are required to cause sterilization, when applied dry and as solutions of various strengths, are averaged, a value of approximately two-thirds of one per cent by weight is obtained. However, sodium fluoride is more effective when applied as a very weak solution.

Sodium Silicofluoride

The effects of sodium silicofluoride on the number and condition of the third stage Sclerostome larvae recovered from faeces are illustrated in Fig. 4.

The action of sodium silicofluoride is similar to that of some of the sulphur and chlorine salts (which will be described in subsequent papers of this series), in that it does not kill many of the larvae until they reach the third stage.

Sodium silicofluoride was tested dry in quantities of 0.05 to 8.0 gm. In one of the cultures in which 0.1 gm. was mixed, a few larvae died after reaching the third stage; in all the cultures in which 0.15, 0.2 or 0.25 gm. was mixed,

the death rate of larvae that reached the third stage was high. From all the cultures in which 0.33 gm. or over was mixed, only dead third stage larvae were recovered. However, the cultures that were treated with 5.0 gm. or less were seldom free of larvae, while in the cultures treated with up to, and including, 0.5 gm. the larvae were numerous. No larvae were isolated

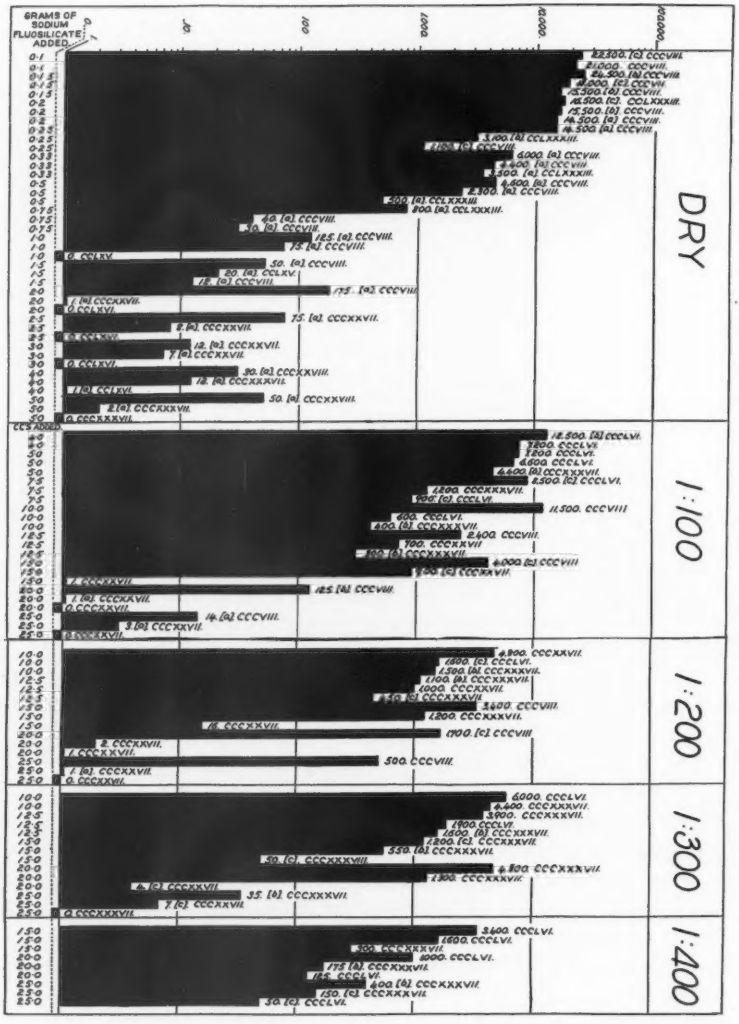

FIG. 4. *Results of tests with sodium fluosilicate, dry and in solution.*

from the cultures treated with 6.0 gm. or over. Since there seems to be no practical method of determining whether the larvae that reached the third stage and then rapidly died would ever have been infective to a suitable host, it is extremely difficult to be certain of the exact effective percentage of dry sodium silicofluoride.

Fig. 4 shows that while there is a distinct tendency for some of the larvae to reach the infective stage and then die when the faeces are treated with sodium fluoride in solution, the tendency is not so marked as when this chemical is applied dry.

Sodium silicofluoride was tested as a 1 : 100 aqueous solution, but at that dilution some sediment remains. The results were irregular; however, since one culture was sterilized by 15.0 cc. and two completely and one almost sterilized by 20.0 cc., it is probable that about 20.0 cc. is the amount required to cause sterilization. This contains 0.2 gm., equivalent to 0.5% of the weight of faeces.

When applied as a 1:200 or as a 1:300 aqueous solution, the exact interpretation of the results again is difficult. When a 1:200 solution was applied, one of three cultures was sterilized by 15.0 cc., and two each by 20.0 and 25.0 cc. When added as a 1 : 300 solution, there were numerous larvae in two cultures treated with 20.0 cc., but 25.0 cc. was effective. If the results are averaged it seems probable that slightly under 25.0 cc. of a 1 : 200 solution and 25.0 cc. of a 1 : 300 solution, or about 0.3% and 0.21% are effective.

Sodium silicofluoride was also tested in 1 : 400, 1 : 500, 1 : 600, and 1 : 800 aqueous solutions. The larger quantities of fluids of all the solutions caused a marked reduction in the number of larvae that reached the third stage; in the stronger of these solutions there was also a tendency for these larvae to die.

If it is considered that 0.33 gm. of dry sodium silicofluoride is effective in sterilizing the 40-gm. cultures, the average sterilizing value of this chemical is slightly under half of one per cent. However, this value may perhaps be too high. This chemical also is most effective when applied as a very weak solution.

Naphthalene

Naphthalene was tested in the faeces and suspended above them. The results obtained are shown in Fig. 5. Quantities of 0.01 to 8.0 gm. were applied.

When the naphthalene was mixed in the faeces the results were more regular. When 0.05 gm. or less was added thousands of larvae survived; in two of the three cultures treated with 0.075 and with 0.1 gm., the number of larvae was considerably reduced. When 0.15 gm. (equal to 0.37%) or more was mixed in the faeces, the cultures were effectively sterilized.

When the naphthalene was suspended above the cultures, it was found that from 0.3 to 1.3 gm. evaporated. One of three cultures was sterilized by 0.1, 0.15, 0.2, and 0.25 gm.; two cultures were sterilized by 0.33 gm., but

thousands of larvae were recovered from other cultures treated with these or with smaller quantities. When 0.5 gm., equivalent to 1.25%, or more was suspended above the faeces they were sterilized against Sclerostomes.

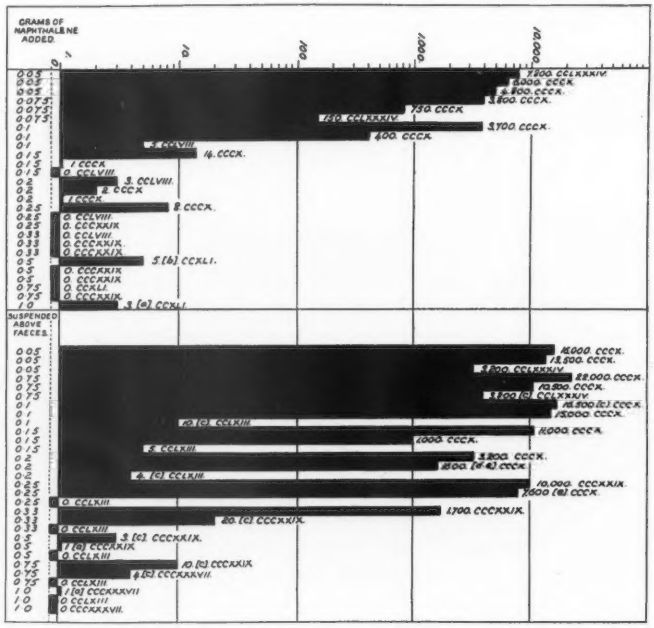

FIG. 5. *Results of tests with naphthalene, mixed in and suspended above the cultures.*

Dichloropentanes

Dichloropentanes, which have a specific gravity of 1.07, were tested in quantities of 0.02 to 25.0 cc. In Fig. 6 are shown the numbers and condition of the Sclerostome larvae which were recovered from the cultures treated with it.

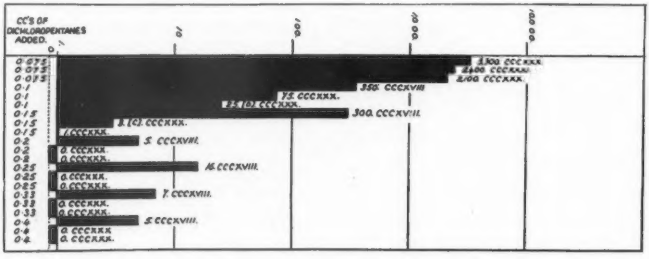

FIG. 6. *Results of tests with dichloropentanes.*

Thousands of larvae were recovered when 0.075 cc. or less was mixed in the faeces. When 0.1 cc. was added, the number of larvae was very considerably reduced and 0.15 cc. sterilized two out of three cultures. The addition of 0.2 cc. (equal to 0.54% of the weight of faeces) or larger quantities, sterilized them against Sclerostomes.

40% Nicotine Sulphate

Fig. 7 illustrates the effect on the Sclerostome larvae of mixing 40% nicotine sulphate, both undiluted and in solution, in faeces. It has a specific gravity of 1.19.

This substance was applied undiluted in quantities of 0.2 to 15.0 cc.; in all cultures treated with 1.5 cc. or less, thousands of larvae reached the third stage; however, in a few cultures some of the larvae subsequently died. When 2.0 cc. was added, 900, 700, and 125 larvae were recovered; 2.5 cc. further reduced the numbers to 500, 300, and 60. From one culture treated with 3.0 cc., 1,000 larvae were isolated, but from the other two cultures only 35 and 30 larvae were obtained; most of the former and all of the latter were dead. The addition of 4.0 cc. practically sterilized the cultures and from the three cultures treated with 5.0 cc. only one larva was obtained. However, from two of the three cultures treated with 7.5 cc., 300 and 50 larvae were obtained, most of the larvae from the former and all from the latter culture being dead; the third culture was free of larvae, as were the cultures treated with larger amounts. If the results obtained are averaged it seems probable that approximately 4.0 cc., or 11.9% by weight of undiluted 40% nicotine sulphate will cause sterilization.

Diluted with twice its volume of water, this substance was tested in quantities of 0.4 to 20.0 cc. When 2.0 cc. or less was applied, thousands of larvae were recovered, including a few that were dead. The results with quantities of 2.5 to 7.5 cc. were irregular. One culture was sterilized by 2.5, 3.0, and 4.0 cc. and in the other culture treated with these quantities there were decreasing numbers of larvae; however, there was a slight increase in numbers in the cultures to which 5.0 cc. was added. The addition of 7.5 cc. was almost effective; greater quantities were uniformly effective. At a dilution of 1 : 2, 7.5 cc. is equivalent to 7.4% by weight.

When diluted with four times its volume of water, the larvae were numerous after treatment with 3.0 cc. or less. When 4.0 cc. or more was added, the number of larvae was considerably reduced, but 15.0 cc., or 8.9%, was necessary to effect complete sterilization. When diluted with eight times its volume of water it was more effective, and 10.0 cc. and over caused sterilization, except in one culture treated with 15.0 cc. Moreover, in the cultures treated with 4.0, 5.0 and 7.5 cc., there was a very marked reduction in the numbers of larvae recovered, and a few cultures were almost sterilized. When diluted at the rate of 1 : 8, 10.0 cc. is equivalent to 3.3% by weight.

Applied in a dilution of 1 : 20 the results again were most irregular; quantities of 7.5 cc. and over caused a considerable decrease in the numbers of larvae, including the sterilizing of one culture by 10.0 cc. and two out of three

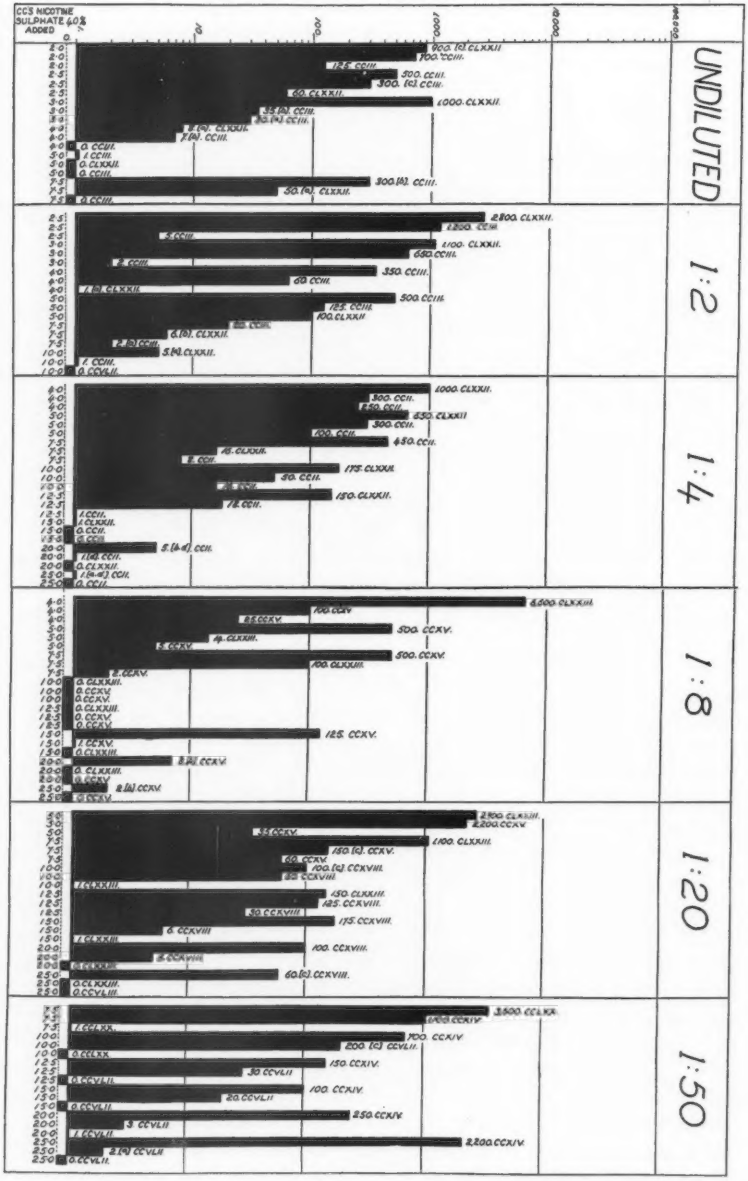

FIG. 7. *Results of tests with 40% nicotine sulphate, undiluted and in solution.*

cultures were sterilized by 15.0, 20.0 and 25.0 cc.; the latter quantity contains the equivalent of 3.5% by weight.

Diluted with 50 times its volume of water the results were equally irregular. One culture was sterilized by 7.5, 10.0, 12.5, and 15.0 cc., and two each by 20.0 and 25.0 cc.; in the other cultures treated by 10.0 cc. and over, the number of larvae was considerably reduced, although from the other cultures treated with 20.0 and 25.0 cc., 250 and 2,200 larvae were recovered. In 25.0 cc. there is the equivalent of 1.46% of 40% nicotine sulphate.

Nicotine sulphate was also tested diluted with 100 and 200 times its volume of water. One culture was sterilized by 15.0, 20.0, and 25.0 cc. of the stronger solution, but in the other cultures there were thousands of larvae.

The results described above suggest that nicotine sulphate is most effective as a medium strength solution, and that the average amount required (of the 40% grade) is about 7% of the weight of faeces.

Ethylenedichloride

Ethylenedichloride, the results with which are illustrated in Fig. 8, has a specific gravity of 1.25.

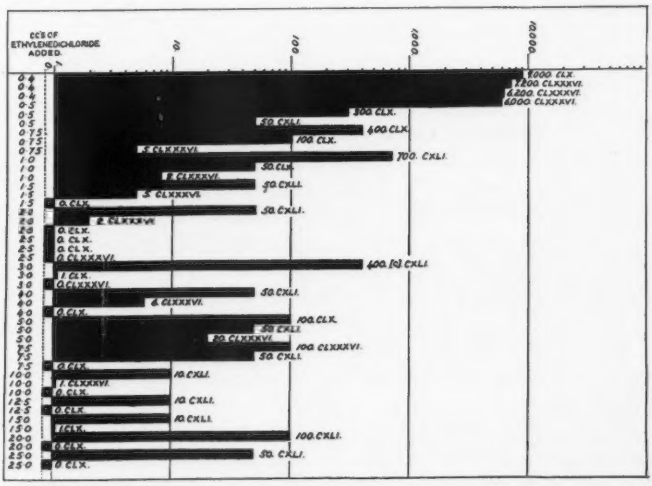

FIG. 8. *Results of tests with ethylenedichloride.*

It was tested undiluted in quantities of 0.25 to 25.0 cc. The results obtained were very irregular. In two of the cultures treated with 0.5 cc. and in all the cultures treated with larger quantities, the number of larvae was considerably reduced, but from many of the cultures treated with up to 25.0 cc. some active larvae were recovered. On an average, the figures suggest that about 1.5 cc. of ethylenedichloride, equivalent to 4.7% of the weight of treated faeces, will sterilize them.

This chemical was also tested as a 1 : 100 aqueous "solution" and as a 1 : 200 aqueous solution, but even when 25.0 cc. was applied several thousand larvae survived.

Chloroform

Fig. 9 shows the results obtained with chloroform. It has a specific gravity of almost 1.5.

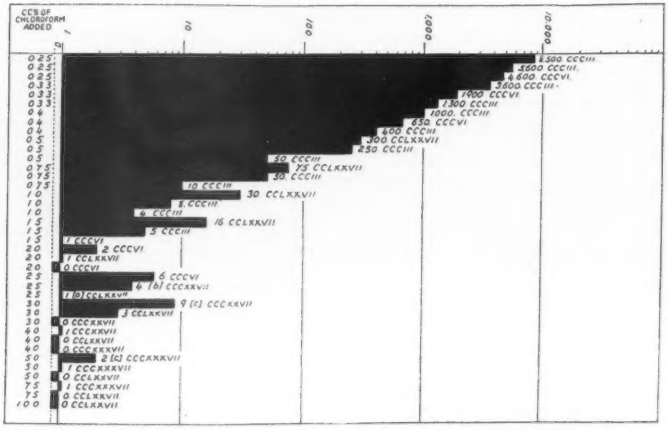

FIG. 9. *Results of tests with chloroform.*

Chloroform was mixed in the faeces in quantities of from 0.1 to 25.0 cc. When 0.33 cc. or less was added, thousands of larvae were recovered from the cultures. With the addition of 0.4 cc. or more, the number of larvae was considerably reduced and became progressively less; however, three and five larvae were recovered from the cultures treated with 15.0 and 20.0 cc. The results indicate that about 1.5 cc., or by weight 5.6%, will sterilize the cultures against Sclerostomes. Chloroform was also tested as a 1:200 aqueous "solution" but was quite ineffective.

Carbon Tetrachloride

The effect of adding carbon tetrachloride to faeces is illustrated in Fig. 10. This chemical has a specific gravity of 1.58. It was added to the cultures in quantities of 0.1 to 25.0 cc. From one culture treated with 0.5 cc., from two with 0.4 and 0.25 cc., and from all cultures treated with 0.33 and 0.2 cc. or less, thousands of active third stage larvae were recovered; from the other cultures the number of larvae was smaller. The addition of 0.75 cc. and over considerably reduced the numbers of larvae, but the results suggest that about 2.0 cc. is necessary to effect sterilization. This quantity is equivalent to just under 8% of the weight of treated faeces.

Trisodium Phosphate

Fig. 11 illustrates the results obtained with trisodium phosphate, which was tested both dry and in solution.

Dry, it was tested in quantities of 1.0 to 10.0 gm. When 3.0 gm. or less was added, thousands of larvae survived; the addition of 4.0 gm. not only considerably reduced the number of larvae that was recovered, but caused a heavy death rate among those which were collected. No larvae were recovered from any of the cultures to which 5.0 gm., or 12.5%, was added, but 175, 75, and 2 larvae, all of which were dead, were recovered from one of each of the cultures treated with 6.0, 7.0, and 8.0 gm.

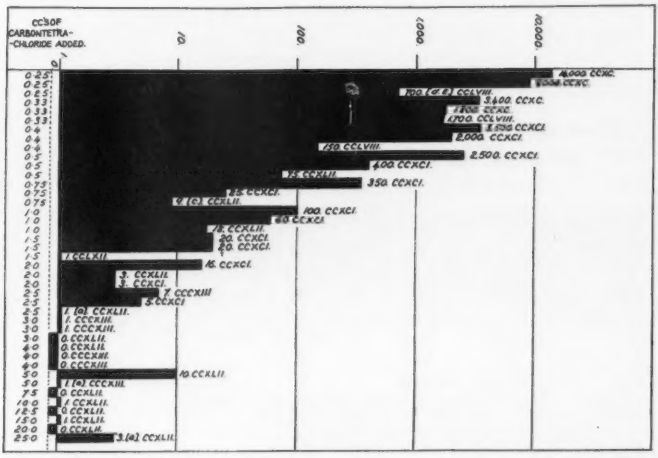

FIG. 10. *Results of tests with carbon tetrachloride.*

Trisodium phosphate was applied as a 1 : 2 aqueous solution in quantities of between 2.0 and 25.0 cc. One culture was sterilized by 10.0 cc., but from the other two treated with this amount or less, thousands of larvae were recovered. The addition of 12.5 cc. greatly reduced the number of larvae and killed the great majority of those recovered. The addition of 15.0 cc. and over sterilized the cultures; in this quantity of fluid there is almost 6.0 gm. of trisodium phosphate, or 15% of the weight of the treated faeces.

Applied as a 1 : 4 aqueous solution, the addition of 12.5 cc. reduced the numbers of larvae in two cultures to 400 and 1,200, and slightly lowered the viability of the larvae in all cultures. Two out of three cultures were practically sterilized by 15.0 and by 20.0 cc.; all were sterilized by 25.0 cc. In the latter quantity there is 6.0 gm., while in 20.0 cc. there is 4.8 gm. or 12% of the weight of treated faeces.

This chemical was added to the cultures as a 1 : 8 aqueous solution in quantities of 5.0 to 25.0 cc. When 15.0 cc. or less was added, thousands of larvae were found. The number of larvae was very considerably reduced in two out of three cultures by 20 cc. The addition of 25.0 cc., containing about 3.0 gm., sterilized one culture, from another 250 larvae were recovered, and from the third 1,500 larvae, but a considerable proportion of these were dead.

A 1 : 20 aqueous solution was also tested, but even 25.0 cc. only reduced the number of larvae to 9,000 and 8,500.

These results suggest that this chemical would have to be added at the rate of about 12.5% by weight to sterilize the faeces against Sclerostomes.

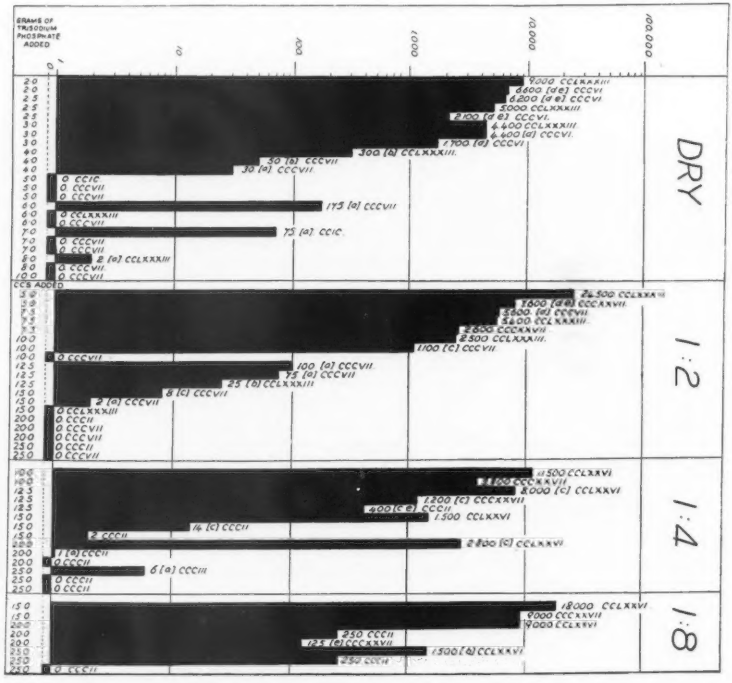

FIG. 11. *Results of tests with trisodium phosphate, dry and in solution.*

Tobacco Dust

Tobacco dust was added dry in quantities of 1.0 to 20.0 gm. The results were irregular and are not illustrated. The number of larvae in one of three cultures was considerably reduced by 4.0, 5.0, and 7.0 gm.; one culture was sterilized by 6.0 gm. The larvae in two out of three cultures were much reduced in numbers by 8.0 and 10.0 gm. The addition of 12.0 gm. sterilized two cultures, and from the third only 150 larvae, including a few dead, were recovered. However, from the cultures treated with 14.0 gm., 5, 200, 250 including many dead, and 8 larvae were isolated. Both 16.0 and 20.0 gm. had the effect of reducing considerably the number of larvae in one culture and sterilizing the other two. These results suggest that the addition of 50% or slightly less of tobacco dust to faeces sterilizes them against Sclerostomes.

Tobacco dust was also tested as an infusion with four times its weight of water; it showed no indication of being effective.

Pyrethrum, Derris, and Hellebore Powders

Pyrethrum powder, derris powder, and white hellebore powder were tested dry, in quantities of 1.0 to 20.0 gm. and as infusions with four times their weight of water in quantities of 2.0, 5.0, 10.0, 15.0, and 20.0 cc. In no case did they have any lethal value.

Ferric Oxide

Brown ferric oxide was applied dry in quantities of 1.0 to 20.0 gm. In the first series of cultures, the largest quantities of ferric oxide appeared to reduce the numbers of larvae, but the confirmatory series showed that this chemical has no lethal value.

Carbon Monoxide

Carbon monoxide was tested by displacing the air in the containers with as much as 500 cc. of gas. The gas was made by dripping formic acid on to warm sulphuric acid, and was collected in a large container, from which it was slowly displaced into the bottom of the vessels containing the cultures. On some of the containers rubber rings were left to retain the gas for 48 hr., 7 days, 14 days, and until the cultures were rebagged a few days before the extraction of the larvae.

Carbon monoxide did not sterilize any cultures with no rubber ring on the jars or on which the ring was left for only 48 hr. Six out of eight cultures were almost or completely sterilized when the rings were left on for 7 or 14 days. But two controls on which the rings were left for 7 and 14 days respectively yielded only 31 and 5 larvae. Furthermore, 12 control cultures were left with the rings on until a few days before the extraction of the larvae; nine yielded no larvae, and from the other three, 25 dead, 40, and 27,000 larvae were obtained. Eleven cultures, treated with 25 to 500 cc. of carbon monoxide, with the rings left on until a few days before the extraction of the larvae, were also free or almost free of larvae. Some of these cultures were kept in the light, others in the dark; no difference was noted.

As 953 control cultures have been made in jars without rubber rings, of which only one has been free of larvae and only three contained under a hundred larvae, it seems evident that it was not the carbon monoxide which sterilized the faeces in these experiments. Probably the cause was the gases generated by the faeces, perhaps associated with lack of oxygen. It seems possible that further work, and tests that are being made in large wooden containers holding nearly a cubic yard of manure, may show that this phenomenon could be used in practice.

Conclusions

The results discussed in this paper show that some chemicals that give off gases are extremely lethal to Sclerostomes, but that others, known to be lethal to other forms of animal life, have a comparatively low lethal value or are useless against Sclerostomes; that some substances, known to be lethal

to some forms of animal life, are useless against Sclerostomes, and that some chemicals, although extremely lethal to Sclerostomes, when added to fresh faeces, do not kill many of the larvae until they reach the third stage.

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